SCHMIDT ET AL. -- 09/806,564
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IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. *(Previously Presented)* A method for characterising a population of parent polypeptides in a sample, which method comprises:

(a) contacting a first portion of the sample with a first sequence-specific cleavage agent to generate polypeptide fragments;

(b) isolating one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the parent polypeptide from which it was fragmented;

(c) identifying the isolated fragments by mass spectrometry;

(d) repeating steps (a)-(c) on a second portion of the sample using a second sequence-specific cleavage agent that cleaves at a different site from the first cleavage agent, wherein the second cleavage agent is different from the first cleavage agent, and wherein the second portion of the sample is separate from the first portion of the sample; and

(e) characterising the parent polypeptides in the sample from the fragments identified in steps (c) and (d).

2. *(Previously Presented)* A method according to claim 1, wherein the step (d) comprises repeating steps (a)-(c) two or more times, each time using a portion of sample which is separate from the previous portions, and each time using a further cleavage agent that cleaves at a different site from the previous cleavage agents.

3. *(Previously Presented)* A method according to claim 1 or claim 2, comprising a further capping step prior to step (a), which capping step comprises reacting the parent polypeptides in the portion of sample with one or more capping agents to introduce capping groups on one or more reactive side chains of the polypeptides.

4. *(Previously Presented)* A method according to claim 3, wherein the capping step and steps (a)-(c) are repeated one, two, or more times, each time using a portion of sample which is separate from the previous portions, and each time introducing capping groups at the

same side chains as the previous capping steps, but using capping groups having different mass than the corresponding capping groups used in the previous capping steps.

5. *(Previously Presented)* A method for characterising a parent polypeptide or a population of parent polypeptides in the sample, which method comprises:

(f) contacting a first portion of the sample comprising one or more polypeptides with a first capping agent in a first capping step to introduce capping groups on one or more reactive side chains of the polypeptides;

(g) contacting the first sample with a sequence-specific cleavage agent to generate polypeptide fragments;

(h) isolating one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the parent polypeptide from which it was fragmented;

(j) identifying the isolated fragments by mass spectrometry;

(k) repeating steps (f)-(j) on a second portion of the sample using a second capping agent that introduces capping groups at the same side chains as the first capping step, but uses capping groups having different mass than the capping groups used in the first capping step, wherein the second portion is separate from the first portion; and

(l) characterising the one or more parent polypeptides in the sample from the fragments identified in steps (j) and (k).

6. *(Previously Presented)* A method according to claim 5, wherein the steps (f)-(j) are repeated two or more times, each time using a portion of sample which is separate from the previous portions, and each time introducing capping groups at the same side chains as the previous capping steps, but using capping groups having different mass than the corresponding capping groups used in the previous capping steps.

7. *(Previously Presented)* A method according to claim 5 or claim 6, wherein the step (k) comprises repeating steps (f)-(j) one, two, or more times, each time using a portion of sample which is separate from the previous portions, and each time using a further cleavage agent that cleaves at a different site from the previous cleavage agents.

8. *(Previously Presented)* A method according to claim 5, wherein the side chains to be capped comprise one or more of the following:

the NH_2 side chain in arginine;

the NH_2 side chain in asparagine;

the NH_2 side chain in glutamine;

the NH_2 side chain in lysine;

the COOH side chain in aspartic acid;

the COOH side chain in glutamic acid;

the OH side chain in serine;

the OH side chain in threonine;

the OH side chain in thyroxine;

the OH side chain in tyrosine; and

the SH side chain in cysteine.

9. *(Currently Amended)* A method according to claim 1, wherein the fragments are isolated by capture on a solid phase, such as ~~DITC glass~~ isothiocyanato glass (or DITC glass) or polystyrene isothiocyanate.

10. *(Previously Presented)* A method according to claim 9, wherein the capture involves covalently bonding the fragments to the solid phase.

11. *(Previously Presented)* A method according to claim 10, wherein the fragments are bound to the solid phase through their N-termini.

12. *(Previously Presented)* A method according to claim 1, wherein each isolated fragment comprises the C-terminus of the parent polypeptide from which it was fragmented.

13. *(Previously Presented)* A method according to claim 1, wherein the cleavage agent employed comprises an endopeptidase or a chemical cleavage agent.

14-17. *(Canceled)*

18. *(Currently Amended)* The method of claim 13, wherein the cleavage agent is at least one compound selected from the group consisting of a Lys-C endopeptidase, a thiocyanate compound, cyanogen bromide, ~~BNPS-skatole~~ 3-bromo-3-methyl-2-(o-nitrophenyl sulphenyl) indolenine-skatole, trypsin, chymotrypsin and/or thrombin.

19. *(Previously Presented)* The method of claim 5 wherein the capping agent is at least one compound selected from the group consisting of an iodacetate compound, an isocyanate compound, a silyl compound, an anhydride, a vinylsulphone compound and a vinyl pyridine derivative.

20. *(Currently Amended)* The method of claim 1 which method further comprises characterizing the one or more parent polypeptides in the sample by comparison with a database based on terminal peptide mass.

21. *(Previously Presented)* A method for detecting the expression of one or more proteins in a tissue, which method comprises characterizing a population of parent polypeptides in a sample of tissue comprising the following steps:

(a) contacting a first portion of the sample comprising one or more parent polypeptides with a first sequence-specific cleavage agent to generate polypeptide fragments;

(b) isolating one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the parent polypeptide from which it was fragmented;

(c) identifying the isolated fragments by mass spectrometry;

(d) repeating steps (a)-(c) on a second portion of the sample using a second sequence-specific cleavage agent that cleaves at a different site from the first cleavage agent, wherein the second cleavage agent is different from the first cleavage agent, and wherein the second portion of the sample is separate from the first portion of the sample; and

(e) characterizing the one or more parent polypeptides in the sample from the fragments identified in steps (c) and (d).

22. *(Currently Amended)* The method of claim 21 which further comprise chracterizing the population of parent polypeptide by comparison with a database based on terminal peptide mass.

23. *(Previously Presented)* A method for assaying for one or more specific polypeptides in a sample, which method comprises characterizing a population of parent polypeptides comprising the following steps:

(a) contacting a first portion of the sample comprising one or more parent polypeptides with a first sequence-specific cleavage agent to generate polypeptide fragments;

(b) isolating one or more polypeptide fragments, each fragment comprising the N-terminus or the C-terminus of the parent polypeptide from which it was fragmented;

(c) identifying the isolated fragments by mass spectrometry;

(d) repeating steps (a)-(c) on a second portion of the sample using a second sequence-specific cleavage agent that cleaves at a different site from the first cleavage agent, wherein the second cleavage agent is different from the first cleavage agent, and wherein the second portion of the sample is separate from the first portion of sample; and

(e) characterizing the one or more parent polypeptides in the sample from the fragments identified in steps (c) and (d); and

(f) determining the presence or absence of said one or more specific polypeptides based on the presence or absence of one or more specific fragments corresponding to said polypeptides.

24. (*Currently Amended*) The method of claim 23, which further comprises characterizing said population of parent polypeptides by determining the presence or absence of said one or more specific polypeptides by comparison to a database based on terminal peptide mass.